In Vitro Effects of Dantrolene on Rat Myocardium

Sylvia Fratea, M.D.,* Olivier Langeron, M.D.,† Yves Lecarpentier, M.D., Ph.D.,‡ Pierre Coriat, M.D.,§ Bruno Riou, M.D., Ph.D.||

Background: Dantrolene is the only known effective treatment for malignant hyperthermia. However, its effects on myocardial contraction and relaxation remain debatable.

Methods: The effects of dantrolene (10^-7 to 10^-5 M) on the contractility of rat left ventricular papillary muscles were investigated in vitro (Kreb-Henseleit solution, 29°C, pH 7.40, 2.5 and 0.5 mM Ca^2+, stimulation frequency 12 pulses/min). The authors studied contraction, relaxation, contraction-relaxation coupling under high and low load, energetics, and postre stimulation. The effects of dantrolene after depletion of catecholamine stores with reserpine also were studied.

Results: Dantrolene induced a moderate concentration-dependent negative inotropic effect at a low calcium concentration (active force at 10^-3 m: 86 ± 14% of control values, P < 0.05), but not at a high calcium concentration. Dantrolene did not significantly modify the curvature of the force-velocity relation, suggesting that it did not modify myocardial energetics. Dantrolene induced no significant lusitropic effect under low load, suggesting that it did not modify calcium uptake by the sarcoplasmic reticulum. Dantrolene did not significantly modify postrest potentiation and postrest potentiation recovery, suggesting that it did not modify maximum capacity of calcium release by the sarcoplasmic reticulum nor its postrest resetting capacity. Reserpine did not modify the myocardial effects of dantrolene.

Conclusions: In rat myocardium, dantrolene did not modify any of the sarcoplasmic reticulum functions tested (uptake, release, postrest recovery). Dantrolene induced a moderate negative inotropic effect, probably mediated by a decrease in transsarcolemmal calcium entry, and this negative inotropic effect was blunted by an increase in calcium concentration. (Key words: Hyperthermia, malignant: dantrolene. Heart, papillary muscle: contractility; relaxation.)

DANTROLENE is the only known effective treatment for malignant hyperthermia.1-5 Dantrolene is a postsynaptic skeletal muscle relaxant that inhibits calcium release from the sarcoplasmic reticulum (SR), possibly by either direct or indirect interaction with the ryanodine receptor.5 However, in more recent studies, researchers provided some convincing evidence of molecular distinction between the Ryanodine receptor and the dantrolene receptor. The effects of dantrolene on intrinsic myocardial contractility remain debatable.5 In experimental studies, researchers reported a slight positive inotropic effect or no significant inotropic effect,7-9 whereas in other studies, a negative inotropic effect was reported.10,11 Some of these studies only explore the effects of low concentrations of dantrolene because of its poor solubility in water. But, although malignant hyperthermia crisis can resolve with small doses (1-2.5 mg·kg⁻¹) of dantrolene when administered early, higher doses are required, leading to high blood concentrations.5 In addition, the effects of dantrolene on myocardial relaxation and energetics remain unknown.

Because of concomitant changes in preload, systemic resistance, and sympathetic activity, the precise effects of drugs on intrinsic myocardial contractility are difficult to assess in vitro. Therefore, we conducted an in vitro study of the effects of dantrolene on rat left ventricular papillary muscle. The experimental model used in the current study enabled us to determine the effects...
of dantrolene on the mechanics and energetics of cardiac muscle. In addition, because the main effect of dantrolene on skeletal muscle is related to its action on SR,\textsuperscript{4,5} we particularly explored the effects of dantrolene on cardiac SR functions. The experimental model we used enabled us to test these functions in a biochemically unaltered preparation.

Materials and Methods

Care of the animals conformed to the recommendations of the Helsinki Declaration, and the study was performed in accordance with the regulations of the official edict of the French Ministry of Agriculture.

Experimental Protocol

After brief anesthesia with ether, the hearts were quickly removed from adult male Wistar rats (Ifa Credo, France), weighing 250–300 g. Left ventricular papillary muscles were excised carefully and suspended vertically in a 200-ml jacketed reservoir with Krebs-Henseleit bicarbonate buffer solution that contained: 118 mm sodium chloride, 4.7 mm potassium chloride, 1.2 mm magnesium sulfate, 1.1 mm dipotassium hydrogen phosphate, 25 mm sodium bicarbonate, 2.5 mm calcium chloride, and 4.5 mm glucose. The Krebs-Henseleit solution was prepared daily with highly purified water (Ecopure, Barnstead/Thermolyne, Dubuque, IA). The jacketed reservoir was maintained at 29°C with a thermostatic water circulator (Polystat 5HP, Bioblock, Illkirch, France) and continuous monitoring of the solution temperature with a temperature probe (Pt100, Bioblock). Preparations were field-stimulated at 12 pulses/min by two platinum electrodes with rectangular wave pulses of 5-ms duration just above threshold. The bathing solution was bubbled with 95% oxygen–5% carbon dioxide, resulting in a pH of 7.4. After a 60-min stabilization period at the initial muscle length at the apex of the length-active isometric tension curve (L\textsubscript{max}), papillary muscles recovered their optimal mechanical performance, which remained stable for many hours. Suitable preparations were selected as described previously.\textsuperscript{12}

Because dantrolene is poorly soluble in aqueous media, we used dimethylsulfoxide as a solvent (Sigma-Aldrich Chemic, L’Isle d’Abeau Chesnes, France). Therapeutic concentrations of dantrolene ranged from 1 to 10 mg·ml\textsuperscript{−1} (0.3–3 \texttimes 10\textsuperscript{−3} m)\textsuperscript{13} Therefore, five concentrations of dantrolene (Sigma-Aldrich Chemic) were tested in a cumulative manner: 10\textsuperscript{−3}, 3 \texttimes 10\textsuperscript{−3}, 10\textsuperscript{−4}, 3 \texttimes 10\textsuperscript{−4}, and 10\textsuperscript{−5} m, with a 15-min period between each additional concentration. In a preliminary study, researchers showed that the effects of the highest concentration of dantrolene remained stable between 15 and 60 min, and that the lowest concentrations had no significant effects. Dimethylsulfoxide alone was tested at five concentrations, corresponding to those tested in the dantrolene group and in the same cumulative manner.

In the first part of this study, four groups of papillary muscles were studied. Two groups were studied at a high extracellular calcium concentration ([Ca\textsuperscript{2+}]\textsubscript{o} = 2.5 mm), one with dantrolene in dimethylsulfoxide (n = 10) and one with dimethylsulfoxide alone (n = 10). Two other groups were studied at a low (0.5 mm) [Ca\textsuperscript{2+}]\textsubscript{o}, one with dantrolene in dimethylsulfoxide (n = 10) and the other with dimethylsulfoxide alone (n = 10). In these two groups, [Ca\textsuperscript{2+}]\textsubscript{o} was decreased from 2.5 to 0.5 mM because rat myocardial contractility is nearly maximum at 2.5 mM and, consequently, it is difficult to quantify a positive inotropic effect without previously decreasing [Ca\textsuperscript{2+}]\textsubscript{o}.\textsuperscript{14} In addition, in rat myocardium, a postrest potentiation study is more sensitive at low [Ca\textsuperscript{2+}]\textsubscript{o},\textsuperscript{15} and a high [Ca\textsuperscript{2+}]\textsubscript{o}, may mask a negative inotropic effect of drugs that interfere with transsarcommal calcium entry.

Because dantrolene is thought to induce sympathomimetic effects related to catecholamine release,\textsuperscript{9} we also tested the effects of dantrolene in rats after depletion of catecholamine stores. For this purpose, rats were treated with 5 mg·kg\textsuperscript{−1} reserpine (Sigma-Aldrich Chemic) that was subcutaneously administered 24 hr before the experiment, as reported previously.\textsuperscript{10} We verified that pretreatment of rats with reserpine completely abolishes the positive inotropic effect of 10\textsuperscript{−3} m tyramine (96 ± 4 vs. 115 ± 15% of control value of active isometric force). In this second part of the study, two groups of papillary muscles were studied. One group (n = 7) was studied at 2.5 mm [Ca\textsuperscript{2+}]\textsubscript{o}, and another group (n = 6) was studied at 0.5 mm [Ca\textsuperscript{2+}]\textsubscript{o}.

Because it was suggested that dantrolene could inhibit calcium channels, we compared the effects of dantrolene to those of nifedipine. Nifedipine (10\textsuperscript{−7} m, Sigma-Aldrich Chemic) in dimethylsulfoxide was studied in two groups of left ventricular papillary muscles. One group (n = 6) was studied at 2.5 mm [Ca\textsuperscript{2+}]\textsubscript{o}, and another group (n = 6) was studied at 0.5 mm [Ca\textsuperscript{2+}]\textsubscript{o}.
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Effects on Diaphragmatic Muscle
To check that dantrolene was pharmacologically active in our experimental model, we tested the effects of dantrolene on rat diaphragmatic muscle (n = 8), using the same experimental protocol. Strips from the ventral part of the costal diaphragm were dissected carefully from the muscle in situ. The muscles were stimulated supramaximally in the tetanus (30 Hz) mode at a stimulation frequency of 12 pulses/min, as reported previously. The following concentrations of dantrolene were tested: 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M. Characteristics of the muscle strips studied were as follows: cross-sectional area: 4.8 ± 2.1 mm^2; initial length (L_{max}): 5.0 ± 0.7 mm^2.

Electromagnetic Lever System and Recording
The electromagnetic lever system has been described previously. Briefly, the load applied to the muscle was determined by means of a servomechanism-controlled current through the coil of an electromagnet. Muscular shortening induced a displacement of the lever, which modulated the light intensity of a photodiode transducer. All analyses were made from digital records of force and length obtained with a computer, as described previously.

Mechanical Parameters
Conventional mechanical parameters at L_{max} were calculated from three twitches. The first twitch was isotonic and was loaded with the preload corresponding to L_{max}. The second twitch was abruptly clamped to zero-load just after the electrical stimulus; the muscle was released from preload to zero-load with critical damping, to slow the first and rapid shortening overshoot resulting from the recoil of series passive elastic components, as reported previously; the maximum unloaded shortening velocity (V_{max}) was determined from this twitch. The third twitch was fully isometric at L_{max}. The mechanical parameters characterizing the contraction and relaxation phases, and the contraction–relaxation coupling, are defined as follows (fig. 1).

Contraction Phase. We determined V_{max} using the zero-load clamp technique; maximum shortening velocity of the twitch with preload only; maximum isometric active force normalized per cross-sectional area (AF); and the peak of the positive force derivative normalized per cross-sectional area (+dF\cdot dt^{-1}). V_{max} and AF tested the inotropic state under low and high loads, respectively.

Relaxation Phase. We determined maximum lengthening velocity of the twitch with preload only and the peak of the negative force derivative at L_{max}, normalized per cross-sectional area (df\cdot dt^{-1}). Nevertheless, because changes in the contraction phase induce coordinated changes in the relaxation phase, these two relaxation parameters cannot assess lusitropy, and, therefore, variations in contraction and relaxation must be considered simultaneously to quantify drug-induced changes in lusitropy. Indexes of contraction–relaxation coupling have, therefore, been developed.

Contraction–Relaxation Coupling. Coefficient R1, the ratio of maximum shortening velocity to maximum lengthening velocity of the twitch with preload, only studied the coupling between contraction and relaxation under low load, and therefore, lusitropy under low load in a manner independent of inotropic changes (fig. 2). Under isotonic conditions, the amplitude of sarcomere shortening is greater than that observed under isometric conditions. Due to the lower sensitivity of myofilament for calcium when cardiac muscle is markedly shortened under low load, relaxation proceeds more rapidly than contraction, apparently due to the rapid uptake of calcium by the SR. Therefore, in rat myocardium, R1 primarily
LOW LOAD

![Graph showing calcium concentration (mM) vs. % of control value for different conditions.](image)

HIGH LOAD

![Graph showing calcium concentration (mM) vs. % of control value for different conditions.](image)

Fig. 2. Effects of calcium on indices of lusitropy under high or low load (n = 8). Under low load, in contrast to both maximum shortening velocity (maxVc) and maximum lengthening velocity (maxVr), R1 is not significantly modified by major inotropic changes induced by decreasing calcium concentration. Under high load, R2 is less modified by major inotropic changes induced by decreasing calcium concentrations than the peak of the positive force derivative (+dF/dt) and the peak of the negative force derivative (-dF/dt). Data are mean percent of control values ± SD.

reflected Ca\(^{2+}\) uptake function of the SR, and R1 is not significantly modified by major inotropic changes induced by decreasing [Ca\(^{2+}\)]\(_i\), (Fig. 2).

Coefficient R2 (+dF/dt\(^{-1}\)/dF\(\cdot\)dt\(^{-1}\)) studied the coupling between contraction and relaxation under high load, and, therefore, lusitropy under high load, which is less dependent on inotropic changes than –dF/dt\(^{-1}\) (Fig. 2). Because of a higher affinity to troponin C for Ca\(^{2+}\) with greater force development and maintained length,\(^{22}\) the time course of relaxation is primarily determined by dissociation of Ca\(^{2+}\) by troponin C rather than by Ca\(^{2+}\) uptake into the SR. Therefore, R2 reflects myofilament calcium sensitivity.\(^{20,25}\) R2 is less modified by major inotropic changes than –dF/dt\(^{-1}\) (Fig. 2). The slight decrease in R2 as [Ca\(^{2+}\)]\(_o\) is decreased (Fig. 2) is consistent with the fact that calcium per se modulates myofilament calcium sensitivity, according to the cooperativity concept.\(^{24}\)

The parameters R1 and R2, which study lusitropy, have been used empirically for many years,\(^{12,20}\) but have been validated recently.\(^{25}\)

**Energetic Parameters**

The force-velocity curve was derived from the peak shortening velocity of 7–9 afterloaded twitches plotted against the total force normalized per cross-sectional area and from that of the zero-load clamp twitch, as reported previously.\(^{26}\) The following energetic parameters were derived from the Hill's hyperbola equation (relation between total force normalized per cross-sectional area and velocity): the peak power output (Emax), and the curvature of the hyperbola (G). The curvature of the hyperbola has been shown to be linked to myothermal efficiency and cross-bridge kinetics; the more curved the Hill's hyperbola (i.e., the higher value of the curvature of the hyperbola [G]), the higher the muscle efficiency.\(^{27,28}\)

**Postrest Potentiation**

Recovery of a stable, reproducible isometric contraction after a rest interval (1 min) was studied to identify the effects of dantrolene on SR functions. During rest in the rat, SR function accumulates additional calcium above and beyond that accumulated with regular stimulation, and the first beat after the rest interval (B1) is more forceful than the last beat before the rest interval (B0). During stimulation of the postrest recovery (B1, B2, B3, . . .), the SR-dependent part of activator calcium decreases somewhat toward a steady state, which is reached in a few beats. Therefore, the effects of dantrolene on the postrest-potentiated contraction may provide insight into its effects on SR functions in a biochemically unaltered preparation. The maximum isometric active force (AF) during postrest recovery was studied at a 0.5 mM [Ca\(^{2+}\)]\(_o\), at a stimulation frequency of 12
pulses/min, and after a 1-min rest duration, and the rate constant $\tau$ of the exponential decay of AF was determined, as described previously. $\tau$ is the number of beats required for the postrest contraction to decay to one tenth of its maximum (R1); it is assumed to represent the time required for the SR to reset itself and was used, therefore, to test SR function.

At the end of the study, the muscle cross-sectional area was calculated from the length and weight of papillary muscle, assuming a density of 1. Shortening and lengthening velocities were expressed in $L_{\text{max}} \cdot \text{s}^{-1}$ and force in mN $\cdot$ mm $^{-2}$.

**Statistical Analysis**

Data are expressed as mean $\pm$ SD. Comparisons of control values between groups were performed using Student's $t$ test or analysis of variance. Comparison of several means were performed using repeated-measures analysis of variance and Newman-Keuls test. The energetic parameters were derived from the Hill's equation using multilinear regression and the least square method, as reported previously. The beat-to-beat decay of active isometric force during postrest recovery was plotted against the number of beats and fitted to an exponential curve, and regression was performed using the least square method, as described previously.

When possible, the concentration–response curve was determined by fitting the data to the sigmoid pharmacologic model from Hill, according to the following equation:

$$\text{Eff}_0 = \text{Eff}_{\text{max}} \cdot C \cdot (C_{\text{SO}} + C)^{-1}$$

in which Eff$_0$ is the observed effect at the C concentration, Eff$_{\text{max}}$ the maximum effect, and C$_{\text{SO}}$ the concentration that results in 50% of Eff$_{\text{max}}$. Iterative nonlinear least square regression curve fitting was used to obtain the best fit (Matlab 1.2e software, The Mathworks, S. Natik, MA). All $P$ values were two-tailed, and a $P$ value of less than 0.05 was required to reject the null hypothesis. Statistical analysis was performed using PCSM software (Deltasoft, Meylan, France).

**Results**

Sixty-five left ventricular papillary muscles were used in the current study. The mean cross-sectional area was $0.73 \pm 0.05$ mm$^2$ (range 0.35–1.05), mean $L_{\text{max}}$ was $4.7 \pm 1.2$ mm (range 3.0–7.5), mean ratio of resting force and total isometric force was $0.14 \pm 0.06$ (range 0.08–0.23), contraction–relaxation coupling under low load (R1) was $0.75 \pm 0.08$ (range 0.55–0.85) at 2.5 mM [Ca$^{2+}$]$_o$, and no significant differences were noted between groups. A decrease in contractility was observed as [Ca$^{2+}$]$_o$ was decreased from 2.5 to 0.5 mM ($n = 32$): decrease in $V_{\text{max}}$ (66 $\pm$ 12% of the value at a [Ca$^{2+}$]$_o$ of 2.5 mm) and AF (63 $\pm$ 15% of the value at a [Ca$^{2+}$]$_o$ of 2.5 mm) were consistent with previous reports.

Dantrolene induced a significant and dose-dependent negative inotropic effect in diaphragmatic muscle. A significant decrease was observed even at a very low concentration ($10^{-8}$ M; 85 $\pm$ 15% of control values). Eff$_{\text{max}}$ was $30 \pm 14$% of control values, and the C$_{\text{SO}}$ was $0.55 \pm 0.74$ $\mu$M.

At a [Ca$^{2+}$]$_o$ of 2.5 mm, dantrolene induced a very modest positive inotropic effect, as shown by the significant increase in AF but not in $V_{\text{max}}$ (fig. 3, table 1). Nevertheless, there were no significant differences when compared with the dimethylsulfoxide group (table 1). Dantrolene did not significantly modify maxVR (data not shown) and contraction–relaxation coupling under low load (R1) (table 1), whereas dimethylsulfoxide slightly increased R1. Dantrolene did not modify $-\text{dF}/\text{dt}^{-1}$ (data not shown) and contraction–relaxation coupling under high load (R2), except at the highest
Table 1. Comparison of the Effects of Dantrolene in Dimethylsulfoxide (DMSO) (n = 10) and those of DMSO Alone (n = 10) on Intrinsic Mechanical Properties of Rat Left Ventricular Papillary Muscles at a High Calcium Concentration (2.5 mM)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>Control Values</th>
<th>Concentrations of Dantrolene or Equivalent Concentrations of DMSO (% of Control)</th>
<th>Between-groups Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^{-3} M</td>
<td>3.10^{-3} M</td>
</tr>
<tr>
<td>Contraction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V_{max} (L_{max}·s^{-1})</td>
<td>Dantrolene</td>
<td>3.29 ± 0.24</td>
<td>103 ± 7</td>
<td>106 ± 10</td>
</tr>
<tr>
<td></td>
<td>DMSO</td>
<td>3.31 ± 0.48</td>
<td>104 ± 5</td>
<td>108 ± 7</td>
</tr>
<tr>
<td>AF (mN·mm^{-2})</td>
<td>Dantrolene</td>
<td>55 ± 26</td>
<td>111 ± 9*</td>
<td>114 ± 14*</td>
</tr>
<tr>
<td></td>
<td>DMSO</td>
<td>50 ± 21</td>
<td>105 ± 8</td>
<td>109 ± 12</td>
</tr>
<tr>
<td>Contraction-relaxation Coupling</td>
<td>R1 (low load)</td>
<td>Dantrolene</td>
<td>0.79 ± 0.04</td>
<td>105 ± 7</td>
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<td></td>
<td>DMSO</td>
<td>0.80 ± 0.05</td>
<td>110 ± 9*</td>
<td>116 ± 11*</td>
</tr>
<tr>
<td></td>
<td>R2 (high load)</td>
<td>Dantrolene</td>
<td>2.49 ± 0.58</td>
<td>101 ± 13</td>
</tr>
<tr>
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<td>DMSO</td>
<td>2.21 ± 0.49</td>
<td>102 ± 12</td>
<td>104 ± 13</td>
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</table>

Data are mean ± SD. No significant difference in control values between groups. AF = isometric active force normalized per cross-sectional area. R1 = ratio of maximum shortening velocity to maximum lengthening velocity, tests the lusitropic effect under low load; R2 = ratio of the peak of positive force derivative to the peak of negative force derivative (-dF·dt^{-1}/-dF·dt^{-1}) tests the lusitropic effect under high load. V_{max} = maximum unloaded shortening velocity.

Table 2. Comparison of the Effects of Dantrolene in Dimethylsulfoxide (DMSO) (n = 10) and those of DMSO Alone (n = 10) on Intrinsic Mechanical Properties of Rat Left Ventricular Papillary Muscles at a Low Calcium Concentration (0.5 mM)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>Control Values</th>
<th>Concentrations of Dantrolene or Equivalent Concentrations of DMSO (% of Control)</th>
<th>Between-groups Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^{-3} M</td>
<td>3.10^{-3} M</td>
</tr>
<tr>
<td>Contraction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V_{max} (L_{max}·s^{-1})</td>
<td>Dantrolene</td>
<td>2.26 ± 0.59</td>
<td>103 ± 6</td>
<td>104 ± 12</td>
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<td>DMSO</td>
<td>2.20 ± 0.45</td>
<td>108 ± 12</td>
<td>111 ± 18*</td>
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<td>AF (mN·mm^{-2})</td>
<td>Dantrolene</td>
<td>32 ± 11</td>
<td>96 ± 9</td>
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<td></td>
<td>DMSO</td>
<td>36 ± 17</td>
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<td>109 ± 19</td>
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<td>Contraction-relaxation Coupling</td>
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<td>Dantrolene</td>
<td>0.78 ± 0.17</td>
<td>104 ± 4</td>
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<td></td>
<td>DMSO</td>
<td>0.71 ± 0.13</td>
<td>98 ± 7</td>
<td>100 ± 10</td>
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<td>R2 (high load)</td>
<td>Dantrolene</td>
<td>1.87 ± 0.37</td>
<td>99 ± 7</td>
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<td>DMSO</td>
<td>2.00 ± 0.43</td>
<td>99 ± 12</td>
<td>102 ± 11</td>
</tr>
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</table>

Data are mean ± SD. No significant difference in control values between groups. AF = isometric active force normalized per cross-sectional area. R1 = ratio of maximum shortening velocity to maximum lengthening velocity, tests the lusitropic effect under low load; R2 = ratio of the peak of positive force derivative to the peak of negative force derivative (-dF·dt^{-1}/-dF·dt^{-1}) tests the lusitropic effect under high load. V_{max} = maximum unloaded shortening velocity.

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Table 3. Comparison of the Effects of Dantrolene in Dimethylsulfoxide (DMSO) (n = 10) and DMSO (n = 8) on Energetic Parameters of Rat Left Ventricular Papillary Muscles at a High (2.5 mM) or Low (0.5 mM) calcium concentration.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>Control (Absolute Values)</th>
<th>Concentrations of Dantrolene or Equivalent Concentrations of DMSO (% of Control)</th>
<th>Between-groups Comparison</th>
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<td>Calcium 2.5 mM</td>
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<td></td>
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<tr>
<td>(E_{\text{max}})</td>
<td>Dantrolene</td>
<td>30.3 ± 11.4</td>
<td>111 ± 6* 118 ± 10* 119 ± 12* 113 ± 14* 98 ± 18</td>
<td>NS</td>
</tr>
<tr>
<td>(L_{\text{max}}) (\text{mN·mm}^{-2}·\text{s}^{-1})</td>
<td>Dantrolene</td>
<td>37.9 ± 4.1</td>
<td>108 ± 10 110 ± 14 111 ± 18 105 ± 19 89 ± 19*</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>2.04 ± 0.47</td>
<td>112 ± 34 117 ± 31 118 ± 25 124 ± 23 131 ± 26</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Dantrolene</td>
<td>2.18 ± 0.41</td>
<td>100 ± 9 101 ± 11 102 ± 11 114 ± 6* 127 ± 11*</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>DMSO</td>
<td></td>
<td></td>
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<tr>
<td>Calcium 0.5 mM</td>
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<tr>
<td>(E_{\text{max}})</td>
<td>Dantrolene</td>
<td>21.1 ± 7.5</td>
<td>97 ± 5 91 ± 11 81 ± 14* 69 ± 15* 50 ± 9*</td>
<td>0.02</td>
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<tr>
<td>(L_{\text{max}}) (\text{mN·mm}^{-2}·\text{s}^{-1})</td>
<td>Dantrolene</td>
<td>15.5 ± 4.7</td>
<td>106 ± 11 116 ± 25 118 ± 31 117 ± 36 91 ± 38</td>
<td>NS</td>
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<tr>
<td></td>
<td>G</td>
<td>2.23 ± 0.51</td>
<td>104 ± 13 114 ± 17 124 ± 33 122 ± 29 107 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Dantrolene</td>
<td>2.18 ± 0.56</td>
<td>104 ± 17 108 ± 30 116 ± 29 113 ± 30 119 ± 24</td>
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<tr>
<td></td>
<td>DMSO</td>
<td></td>
<td></td>
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</table>

Data are mean ± SD. No significant difference in control values between groups. \(E_{\text{max}}\) = peak power output; \(G\) = curvature of the force–velocity hyperbola. * \(P < 0.05\) versus control values.

into account the negative inotropic effect observed with dantrolene. Dantrolene (10\(^{-3}\) M) induced a negative inotropic effect comparable to that obtained by decreasing calcium concentration from 2.5 to 0.75 mm (67 ± 18% vs. 70 ± 11% of control values, NS). In these conditions, the decrease in R2 induced by dantrolene was not significantly different from that obtained by decreasing calcium concentration (84 ± 11% vs. 88 ± 7% of control values, NS).

At a \([Ca^{2+}]_{o}\) of 2.5 mm, the force–velocity relation was not modified by dantrolene, as shown by the non-significant changes in peak power output and in the curvature of the force–velocity hyperbola (table 3). At a \([Ca^{2+}]_{o}\) of 0.5 mm, the negative inotropic effect of dantrolene was confirmed by the significant decrease in peak power output. Nevertheless, this negative inotropic effect was associated with no significant changes in the curvature of the force–velocity hyperbola (table 3).

Reserpine was administered in two groups of rats to deplete catecholamine stores. At the concentration of dantrolene that was associated with the greatest positive inotropic effect at 2.5 mm calcium (i.e., 3 · 10\(^{-3}\) M), reserpine treatment did not modify the inotropic effect (AF: 106 ± 13% vs. 114 ± 14%, NS) and the lusitropic effect (R2: 85 ± 5% vs. 85 ± 11%, NS) of dantrolene under high load. At the concentration of dantrolene associated with the greatest negative inotropic effect at 0.5 mm calcium (i.e., 10\(^{-3}\) M), reserpine treatment did not modify the inotropic effect (AF: 73 ± 11% vs. 67 ± 18%, NS) and the lusitropic effect (R2: 97 ± 9% vs. 99 ± 13%, NS) of dantrolene under high load.

At a low \([Ca^{2+}]_{o}\) (0.5 mm), 10\(^{-7}\) M nifedipine induced a moderate negative inotropic effect comparable to that of 10\(^{-3}\) M dantrolene. Increasing \([Ca^{2+}]_{o}\) to 2.5 mm blunted this negative inotropic effect, as observed with dantrolene (fig. 4). Nifedipine did not significantly modify R1 (105 ± 11% of control values) and R2 (97 ± 13% of control values).

Postrest recovery was studied after and during an isometric beating period. In control conditions, the first beat (B1) after rest interval was potentiated as compared with the beat before rest (B0), providing a ratio B1/B0 of 1.34 ± 0.15, which was not significantly different between groups and consistent with previous reports. As shown in figure 4, dantrolene did not significantly modify the ratio B1/B0, whereas dimethylsulfoxide alone slightly decreased it at high concentrations. However, no significant difference was noted between the two groups (fig. 5).

The decay of mean active isometric force during the postrest recovery period is shown in figure 6. This decay fitted well to an exponential curve (0.95 < R < 0.99), and the control values of the rate constant (\(\tau\) = 3.6 ± 1.5 beats) were not significantly different between groups and were consistent with our previous studies. Even at 10\(^{-3}\) M, dantrolene in dimethylsulfoxide (3.6 ± 0.6 vs. 3.6 ± 1.0 beats, NS) and dimethylsulfoxide alone (3.6 ± 1.9 vs. 3.7 ± 1.3 beats, NS) did not significantly modify \(\tau\) (fig. 6).

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Discussion

We studied the effects of dantrolene on the intrinsic contractility of isolated rat left ventricular papillary muscle at different calcium concentrations. The main results were that dantrolene did not modify any of the SR functions tested, and that the inotropic effect of dantrolene depends on calcium concentration.

At 2.5 mM calcium, dantrolene did not significantly modify the peak power output, whereas it significantly decreased it at 0.5 mM (table 3). Nevertheless, whatever the effect on the peak power output, dantrolene did not significantly modify the curvature (G) of the force-velocity curve (table 3). The curvature of the force-velocity curve has been shown to be linked to myothermal economy and cross-bridge kinetics, the more curved the hyperbola (i.e., the higher the value of G), the higher the muscle efficiency. These results show that dantrolene did not significantly modify the energetics of rat myocardium.

Whatever the calcium concentration, dantrolene did not modify contraction-relaxation coupling under low load (R1). Under isotonic conditions, the amplitude of sarcomere shortening is greater than that observed in isometric conditions, and the time course of isotonic relaxation occurs earlier and more rapidly than that of isometric relaxation, partly through two mechanisms: the easier removal of calcium from troponin C, due to a decrease in myofilament calcium sensitivity, and the rapid uptake of calcium by the SR. Under low load, SR appears to play a major role in the regulation of the time course of isotonic relaxation. Our results, therefore, suggest that dantrolene did not modify the uptake of calcium by SR.

The characteristics of force postrest recovery in the rat ventricle have been studied extensively and are shown in figure 5. The first beat of postrest recovery (B1) is more dependent on SR than subsequent beats and the beat before rest. This postrest potentiation is abolished by ryanodine, a specific inhibitor of SR function, which locks the calcium release channels of the terminal cisternae in the open state. The potentiated contraction B1 depends on the capacities of the SR to progressively load more and more calcium during the rest period and to release calcium. In our study, dantrolene did not decrease B1 and the ratio B1/B0 (fig. 5). These results suggest that dantrolene did not impair either SR calcium release function or the capacity of SR to load calcium during the rest period.

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![Graph showing effects of dantrolene on percent of B0](image)

Fig. 6. Effects of dantrolene (10^{-3} M, n = 8) on the decay of mean active isometric force during the postrest recovery period. Data are expressed as mean percent of B0 (the beat before rest) ± SD and plotted on a semilogarithmic scale. No significant differences in the recovery time constant (τ) were observed.

The decay of force during the postrest recovery has been shown to be exponential, and the rate constant τ has been assumed to represent the time required for the SR to reset itself, and was therefore used to test some of the SR functions.\textsuperscript{12,15,29} No significant changes in τ were observed with dantrolene (fig. 6), suggesting that dantrolene did not modify this SR function. Therefore, whatever the SR function tested (uptake, release, postrest recovery), dantrolene, even at a very high concentration, did not modify any of these functions. These results contrast with the effects of dantrolene on skeletal muscle.\textsuperscript{3-5} It was suggested that dantrolene interacts either directly or indirectly with the ryanodine receptor.\textsuperscript{5} Many differences have been noted between the cardiac and skeletal ryanodine receptors, which are distinct proteins whose amino-acid sequences are 66% identical, encoded by different genes, and localized on different chromosomes.\textsuperscript{31} The cardiac ryanodine receptor is more sensitive to activation by Ca\textsuperscript{2+} and is sensitive to inhibition by Mg\textsuperscript{2+} and ruthenium red.\textsuperscript{31} In addition, in a recent study, convincing evidence was provided of a molecular distinction between dantrolene and ryanodine receptors.\textsuperscript{9} Our results suggest that there is no physiologically active dantrolene receptor in cardiac SR comparable to that present in skeletal SR. This absence of effect on SR functions does not preclude a complex interaction with pharmacologic agents known to interact with SR function. Indeed, it was shown that dantrolene inhibits caffeine-induced spontaneous contraction in rat myocardium.\textsuperscript{32}

R2 tested contraction–relaxation coupling under high load. Dantrolene significantly decreased R2, suggesting that it decreased myofilament calcium sensitivity, either directly or indirectly. Dantrolene was reported to induce catecholamine release,\textsuperscript{8} which would, in turn, decrease Ca\textsuperscript{2+} myofilament sensitivity, mediated via cyclic adenosine monophosphate and protein kinase A.\textsuperscript{33} However, the absence of any change in effect after reserpine pretreatment suggests that catecholamine release does not play a role in the positive inotropic and lusitropic effects observed.

At a [Ca\textsuperscript{2+}]c of 0.5 mM, dantrolene induced a concentration-dependent negative inotropic effect. Because dantrolene did not modify SR function, two mechanisms could explain this negative inotropic effect: a decrease in transsarcolemmal calcium entry or a decrease in myofilament calcium sensitivity. The fact that the negative inotropic effect of dantrolene was completely abolished at a high [Ca\textsuperscript{2+}]c (table 1, fig. 5), as observed with nifedipine (fig. 4), a calcium channel blocker, greatly suggests that dantrolene decreased transsarcolemmal calcium entry. This hypothesis is consistent with some previous experimental reports.\textsuperscript{9,10} However, it should be emphasized that this negative inotropic effect was moderate and occurred only at high, supratherapeutic concentrations of dantrolene.

The following points must be considered in the assessment of the clinical relevance of our results. First, because this study was conducted in vitro, it dealt only with intrinsic myocardial contractility. Observed changes in cardiac function after in vivo dantrolene administration may also depend on modifications in venous return, afterload, and reflex regulatory and com-
pensatory mechanisms. Nevertheless, the lack of significant inotropic effect at therapeutic concentrations in vitro are consistent with the moderate cardiovascular effects of dantrolene in vitro. Second, because this study was conducted at 29°C, at a low-stimulation frequency, and in the rat, which differs somewhat in its cardiac behavior from other species, including humans, the implications for clinical practice are necessarily limited. Nevertheless, our study provides a potential explanation for the discrepancies between previous in vitro studies of dantrolene: the inotropic effect of dantrolene depends on calcium concentration and, therefore, probably on the contribution of sarcoplasmic calcium entry to the myocardial contractility, which is known to differ from one species to another. In addition, our study emphasizes the need to study different calcium concentrations in the rat myocardium to understand the inotropic effect of drugs.

In conclusion, in this study, conducted on isolated rat left ventricular papillary muscle, dantrolene induced a moderate negative inotropic effect at a low calcium concentration, probably related to a decrease in transsarcolemmal calcium entry. This negative inotropic effect was completely abolished by high calcium concentration. Even at highly supratherapeutic concentrations, dantrolene did not modify any of the SR functions tested, which contrasts with its effects on skeletal muscle. Finally, the effective dantrolene concentration required to depress myocardium, even in low Ca²⁺, was substantially greater than that observed clinically in patients given an oral loading dose.

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